Influence of Systemic Hypotension on Skeletal Muscle Ischemia-Reperfusion Injury After 4-Hour Tourniquet Application

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OBJECTIVE: Tourniquet use for extremity hemorrhage control is common in military trauma. Tourniquet use may be accompanied by systemic hypotension, but this phenomenon has not been studied. We aimed to define the muscle effects of the combined insult of tourniquet-induced skeletal muscle ischemia-reperfusion injury (I-R) and hemorrhagic hypotension.

DESIGN: After a 33% carotid arterial hemorrhage, Sprague-Dawley rats underwent 240-min hindlimb ischemia induced by pneumatic tourniquet. Control animals were not hemorrhaged. No resuscitation was given. After tourniquet release, muscles were reperfused for 120 min and then dissected. Weights and mitochondrial viability assays (nitroblue tetrazolium method) were performed on the plantaris (PL), and soleus (SO). Histologic analysis was performed on the PL and SO. Muscle edema is expressed as the ratio of tourniquet limb to contralateral limb muscle weight.

SETTING: Animal laboratories of the United States Army Institute of Surgical Research.

STUDY ANIMALS: Twelve Sprague-Dawley rats.

RESULTS: The mean arterial pressure of hemorrhaged animals was 38 $\pm$ 3 mm Hg before tourniquet placement and 86 $\pm$ 4 mm Hg before release, both significantly ($p < 0.05$) lower than controls at the same time points. Pre- tourniquet mortality was 38% with hemorrhage and 0% without. All muscles experienced edema, with weight ratios greater than 1. The PL experienced significantly ($p < 0.05$) less edema with hemorrhage. Viability was unaffected by hemorrhage in all muscles, as was tissue inflammation. No differences in inflammation were observed with hemorrhage.

CONCLUSIONS: Systemic hypotension modulates the impact of 4 hours of tourniquet ischemia by decreasing muscle edema but minimally altering measures of muscle viability. Compartment anatomy and muscle fiber type both influence muscle response to the combined insult of hypotension and I-R. In this model, hypotension did not worsen the skeletal muscle I-R observed after the use of a tourniquet for 4 hours. (J Surg 64:273-277. Published by Elsevier Inc. on behalf of the Association of Program Directors in Surgery.)

KEY WORDS: rat, edema, muscle injury, hemorrhage, viability, fiber type, trauma

COMPETENCY: Medical Knowledge

Wounds to the extremities are the most frequently encountered in combat, accounting for between 60% and 80% of injuries on the modern battlefield.[1-5] These injuries are frequently severe and are often accompanied by major vascular damage, which makes them a leading contributor to preventable battlefield mortality.[6,7] Although uncommonly employed in civilian trauma, the liberal use of tourniquets under combat conditions is a vital lifesaving protocol for the prevention of exsanguination from severe battlefield extremity injuries.[8] Despite the presence of severe wounds, limbs with combat injuries requiring tourniquets are not universally doomed to amputation and limb salvage is frequently attempted.[9]

Tourniquets are not completely benign interventions, however, and their use may result in potentially significant complications. Damage to a limb to which a tourniquet has been applied may occur as a direct result of the compressive force of the device or indirectly via the occlusion and subsequent restoration of distal tissue perfusion. Ischemia-reperfusion injury (I-R) is the most significant aspect of the indirect injury. Skeletal muscle is particularly susceptible to this phenomenon, and this tissue can demonstrate significant edema and loss of cellular viability after an ischemic period of 2 hours or more.[10]

The use of tourniquets in extremity trauma remains a highly controversial issue, and there is little available published scientific literature regarding the complications of tourniquet use as

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it applies to hemorrhage control in military or civilian extremity trauma.[11,12] Nearly all of the experimental literature on the subject of tourniquet-induced skeletal muscle I-R models the use of tourniquets for elective orthopedic operations. Tourniquet use in the presence of severe extremity trauma typically occurs in the setting of a significant loss of blood from the injured extremity and possibly other sites, frequently resulting in systemic hypotension. The effect of the combination of tourniquet-induced I-R and systemic hypotension on skeletal muscle has not been experimentally investigated in a model combining the 2 insults. Although skeletal muscle seems to escape significant damage in the setting of hemorrhage alone,[13] it is possible that the combination of significant systemic hypotension and tourniquet application may interact in the setting of I-R.

Introductory experiments at our institution using our model confirmed the lack of a deleterious effect of systemic hypotension alone on skeletal muscle. The objective of this study was to determine the relationship of pre-tourniquet hypotension on acute (2 hour reperfusion) skeletal muscle I-R in order to better define the phenomenon in the context of the use of tourniquets after traumatic hemorrhage. We hypothesized that pre-tourniquet hemorrhage would result in less edema during reperfusion and that muscles of varying predominant fiber-type compositions would be differentially affected by hypotension.

MATERIALS AND METHODS

Animal Care

All animal protocols were approved by the United States Army Institute of Surgical Research animal care and use committee. Animals were housed in a vivarium approved by the Association for the Assessment and Accreditation of Laboratory Animal Care. Animals were provided with food and water ad libitum before and after all procedures. All procedures were performed under 1.5% to 2.5% isoflurane anesthesia, which was adjusted to maintain a surgical plane. Postprocedural pain was controlled with buprenorphine, 0.1 mg/kg, administered via deltoid intramuscular injection.

Surgical Procedures

Twelve male Sprague-Dawley rats weighing 399 ± 46 g were anesthetized and placed supine on a warm water flow temperature-regulated bed (EX-212; Euthanex Corp., Palmer, Pennsylvania), with an electronic rectal temperature probe (Physiotemp Instrument, Inc., Clifton, NJ) continuously recording core temperature, which was kept at 37 ± 1°C. The bilateral hindlimbs were shaved, and animals underwent open cannulation of 1 carotid artery with a PE50 polyethylene catheter. The cannula was coated with an anticoagulant (TDMAC-Heparin, 2%; Polysciences, Inc., Warrington, Pennsylvania) with a 23-gauge tubing adapter for syringe attachment. Treatment animals (n = 6) then underwent a 33% arterial hemorrhage at a rate of 1 ml/minute. The carotid cannula was used to provide continuous measurement of mean arterial pressure (MAP) throughout the experiment. Point values were recorded for individual animals at specific times throughout the experiment. Control animals (n = 6) underwent identical procedures but were not hemorrhaged. No resuscitation was provided to any animals.

Exsanguination of the experimental leg was performed by elevation above the level of the heart for 5 minutes. One minute after the completion of hemorrhage, a pneumatic digit tourniquet (model DC1.6; D.E. Hokanson, Inc., Bellevue, Washington) attached to a tourniquet regulation system (model E20; D.E. Hokanson, Inc.) and air source (model AG101; D.E. Hokanson, Inc.) was placed as proximal as possible around the limb and inflated to a pressure of 250 mm Hg for a period of 4 hours. The use of 250 mm Hg was based on pilot studies using orthogonal polarizing spectral imaging (Cytometrics, Bridleways, England) to image blood flow. These studies found that 220 mm Hg effectively eliminated distal microvacular blood flow; we then chose to add a safety factor of 30 mm Hg to this. After the 4-hour limb ischemic time, the tourniquet was released and the animal remained supine and anesthetized for 2 hours; at which time, euthanasia and bilateral hindlimb dissection were performed.

Endpoint Determination

Results were obtained on 2 muscles from each hindlimb. As muscle fiber-type composition has been demonstrated to influence response to I-R, we chose to examine the plantaris (PL), a predominantly fast-twitch muscle, and the soleus (SO), the only predominantly slow-twitch muscle in the rat hindlimb. The muscles of the tourniquet and contralateral limbs were individually dissected and removed with their tendons. Whole muscle weights were obtained on a microbalance (MT-5; Mettler Toledo, Inc., Columbus, Ohio). Muscle viability was determined on a proximal portion of the muscle using the spectrophotometric nitroblue tetrazolium (NBT) reduction technique according to the method of Powell et al.[14]

Statistical Analysis

Muscle weights were standardized within each animal to the contralateral (control) limb for statistical analyses. The ratios of tourniquet to contralateral limb muscle weight were calculated for each muscle by dividing tourniquet limb values by the corresponding contralateral muscle values. NBT was calculated as mg NBT per mg protein in the muscle to compensate for differing sample masses. Group mean values for experimental conditions (hemorrhage versus no hemorrhage) were compared using the Student t-test. In cases where data were not normally distributed, the Mann-Whitney rank sum test was used for comparisons of group medians. Differences were considered to be significant at p less than 0.05. All data are presented as mean ± standard error of the mean.

RESULTS

Hemorrhage

The mean volume of hemorrhage in was 10 ± 0.3 ml, and the hemorrhage correspondingly lasted approximately 10 minutes per animal. The baseline MAPs of the hemorrhaged (92 ± 4 mm Hg) and non-hemorrhaged (95 ± 3 mm Hg) animals were not different. Hemorrhage resulted in a significant drop in MAP to 38 ± 9 mm Hg at the time of tourniquet placement. At the same time point, the MAP of the non-hemorrhaged animals remained 95 ± 3 mm Hg ($p < 0.001$). The hypotensive group gradually compensated during the limb ischemic period, and immediately before tourniquet release, the hemorrhaged animals' mean MAP was 86 ± 4 mm Hg, which was significantly lower than that of the non-hemorrhaged animals at 98 ± 6 mm Hg ($p = 0.048$). After 2 hours of reperfusion, the MAP of hemorrhaged animals remained significantly lower at 82 ± 3 mm Hg than that of non-hemorrhaged animals at 97 ± 4 mm Hg ($p = 0.02$).

Muscle Edema

No significant differences were found in group mean contralateral muscle weights between hemorrhaged and non-hemorrhaged animals at either time point, which indicates a lack of an independent effect of hemorrhage on muscle weight. All tourniquet limb muscles experienced edema, with tourniquet limb-to-contralateral limb muscle weight ratios greater than 1. Hemorrhage resulted in decreased mean weight ratios in all muscles, but the difference was significant only in the PL (1.15 ± 0.08 vs 1.56 ± 0.07, $p = 0.01$) and SO (1.16 ± 0.05 vs 1.36 ± 0.03, $p = 0.04$) (Fig. 1).

Muscle Viability

No significant differences were found in group mean contralateral muscle NBT reduction between hemorrhaged and non-hemorrhaged animals at either time point, which indicates a lack of independent hemorrhage effect on this measure of viability. All tourniquet limb muscles experienced a decrease in viability compared with the contralateral, with tourniquet-to-contralateral muscle viability ratios less than 1. Both muscles experienced a decrement in NBT reduction with hemorrhage, although none of the differences was significant between hemorrhaged and non-hemorrhaged groups (Fig. 2).

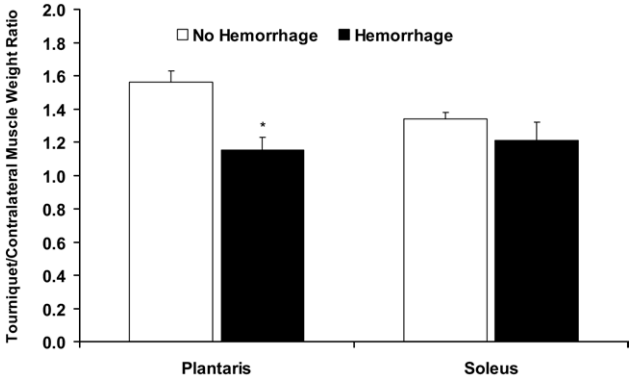

FIGURE 1. Tourniquet-to-contralateral limb muscle weight ratios. Error flags represent the standard error of the mean. Muscle weight in the tourniquet limb is expressed as a fraction of the control (contralateral) limb; ratios greater than 1.0 indicate edema in the tourniquet limb muscles. Pre-tourniquet hemorrhage-induced hypotension resulted in decreased edema in all muscles, significantly (*$p < 0.05$) in the Plantaris (PL).

DISCUSSION

The combined influence of systemic hypotension and tourniquet-induced I-R on skeletal muscle has not been previously reported. We have presented the first in a series of studies designed to characterize the pathology of hemorrhage and I-R as stressors to skeletal muscle. Our results indicate that after 2 hours of reperfusion, significant hypotension resulting from hemorrhage has the broad effect of reducing muscle edema in certain muscles but has a negligible effect on muscle viability.

In this model of tourniquet-induced I-R, pre-tourniquet systemic hypotension affected skeletal muscles differently depending on their predominant muscle fiber type composition. Various small animal studies have investigated the differential sensitivities of skeletal muscle fiber types to ischemia and reperfusion. The effects observed in different models are not highly consistent, although muscles predominantly composed of slow-twitch (type I, oxidative) fibers seem to be relatively resistant to I-R-induced acute decreases in functional parameters, metabolic derangements, and inflammation.[15-18] The reason for the differences between fiber types in vulnerability to I-R remains to be definitively elucidated; however, the potential of predominantly oxidative muscles to metabolically process reactive oxygen species (ROS) such as O^- and NO^- believed to be responsible for I-R-mediated microvascular injury is a potential explanation.[19,20] Another explanation is the higher constitutive level of heat shock proteins, including HSP70, heme oxygenase, and beta-crystalin present in type I muscles. These proteins have been shown to play beneficial roles in reducing I-R-mediated cellular damage.[21]

In the rat, the PL is composed predominantly of type II, fast-twitch muscle fibers. These fibers are principally glycolytic in their metabolism. The SO is composed predominantly of type I, slow-twitch muscle fibers, which are principally oxidative.[22] In our experiment, hemorrhage-induced hypotension led to significantly decreased edema (as evidenced by decreased wet weight ratios) in the PL.

It has been demonstrated that I-R-induced muscle damage is reduced when restoration of blood flow is controlled to blunt the hyperemic response during early reperfusion.[23-25] This effect is believed to be mediated via a reduction in the early reperfusion oxidative burst and a resultant decrease in microvascular damage by ROS and associated metabolites, which are associated with capillary endothelial injury.[26,27] One likely mechanism of the decreased edema observed with hypotension in the PL was hemorrhage-induced skeletal muscle vasoconstriction. This effect may have blunted the normal hyperemic

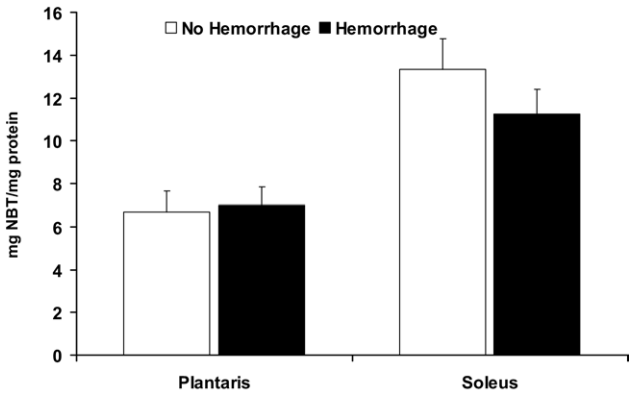

FIGURE 2. Tourniquet-to-contralateral limb muscle viability. Error flags represent the standard error of the mean. Muscle viability as determined by reduction of NBT is expressed as a fraction of the total muscle protein concentration. Larger numbers indicate greater viability. Pre-tourniquet hemorrhage-induced hypotension resulted in no significant changes in viability. Pooled mean NBT reductions in the contralateral muscles were 16.0 ± 1.2 for the plantaris and 17.0 ± 1.1 for the soleus.

response after ischemia and lessened the damaging effects of ROS on the microvasculature. With a more intact microvasculature, capillary hyperpermeability was reduced and muscle edema less pronounced. The predominantly oxidative SO remained resistant to changes in edema with hemorrhage, reflecting its inherent ability to blunt the ROS-mediated microvascular insult of early reperfusion.

All muscles in this study experienced viability loss after tourniquet-induced I-R. As might be expected from their predominant fiber-type composition differences, muscle viability distal to the tourniquet in both hemorrhaged and non-hemorrhaged animals was greater in the SO than in the PL. This result again reflects the inherent protective properties that type I oxidative muscle fibers possess in the setting of I-R.

A relationship between edema and muscle viability has not been directly elucidated in the I-R literature, and our results fail to indicate a relationship between our index of muscle viability and the presence of edema. These results do indicate an effect of pre-tourniquet systemic hypotension on tourniquet-induced skeletal muscle I-R, which decreases edema in the acute phase in the predominantly fast-twitch PL. Muscle viability is not significantly affected in the muscles suffering the most edema, which calls into question a relationship between edema and muscle viability in this model.

This report represents the first attempt to elucidate the effect of systemic hypotension caused by hemorrhage on tourniquet-induced skeletal muscle I-R. Although novel, the experiment reported has limitations that bear mentioning. Although a significant degree of hypotension was reached during the initial phase of the experiment, we lack data such as pH and serum lactate from these animals. Such data could definitively determine whether the animals were in hemorrhagic shock and the specific physiology of this shock during the experiment. The fact that a difference in muscle edema response to I-R was observed with and without hemorrhage does suggest that the hemorrhage protocol in this model was significant enough to produce a local effect. It is unknown, however, whether a different hemorrhage protocol might produce a difference in muscle viability, which was not observed with this protocol.

This model produced a combined insult of hemorrhage-induced systemic hypotension and 4 hours of tourniquet limb ischemia. Hemorrhage resulted in decreased muscle edema but unaltered measures of muscle viability. Muscle fiber-type composition seemed to play a role in the response to the combined insult of hypotension and I-R. These results represent the initial phase of our understanding of a complex clinical and pathophysiologic interaction. Many questions are raised by these data, and many aspects of the interaction are available for study. The functional consequences of the combined skeletal muscle insult in this model are as yet undefined. Future experiments are slated to examine these consequences through the use of an in situ muscle functional assessment using muscle contractile properties as endpoints. Also unstudied are the potential effects of fluid resuscitation and post-hemorrhage treatments on the pathophysiologic interaction elucidated here. We plan to investigate numerous resuscitation fluids and protocols to evaluate their effect on the physiologic, anatomic, and functional consequences of this model. Treatments, both pharmacologic (such as anti-inflammatory compounds) and procedural (such as fasciotomies), will also be studied.

The use of tourniquets in cases of severe extremity trauma with significant hemorrhage will continue in military situations, and limb salvage will continue to be a priority in casualties so injured. Continued investigation into the unique physiologic aspects of tourniquet use in extremity trauma should remain a research priority.

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